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INHIBITION OF LIVER ENDOPLASMIC RETICULUM CALCIUM PUMP BY CCl_4 AND RELEASE OF A SEQUESTERED CALCIUM POOL*

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Abstract—One of the earliest effects observed in rat liver after CCl_4 administration is inhibition of an ATP-dependent calcium pump found at the endoplasmic reticulum. This report confirms that the amount of calcium associated with the microsomal fraction is reduced after CCl_4 administration and, for the first time, demonstrates time-, dose-, and metabolism-dependent relationships between inhibition of the liver microsomal calcium pump and the amount of calcium found in the microsomal fraction. Furthermore, release of calcium from the endoplasmic reticulum is shown to cause activation of a cytoplasmic enzyme that responds to increases of ionized calcium, glycogen phosphorylase. This suggests that the endoplasmic reticulum calcium pump sequesters an intracellular pool of calcium within the endoplasmic reticulum. This pool of calcium may be released into the cytoplasm as a consequence of inhibition of the calcium pump by CCl_4.

Microsomal fractions isolated from a number of nonmuscle cells and tissues have an energy-dependent calcium uptake system [1–3]. Several investigators have noted similarities between these calcium uptake systems and the skeletal muscle sarcoplasmic reticulum calcium pump. These similarities have led to the suggestion that the microsomal calcium uptake system is an endoplasmic reticulum (ER) calcium pump which functions to sequester cytoplasmic calcium and thus participates in regulation of ionized calcium in cytoplasm [4, 5]. However, there is little evidence demonstrating that a pool of calcium sequestered by the microsomal uptake system can be released by a hepatotoxin.

Reynolds *et al.* [6] demonstrated that *in vivo* administration of a hepatotoxin such as CCl_4 results in a prompt, extensive loss of liver endoplasmic reticulum associated calcium. This change in microsomal (endoplasmic reticulum) calcium content occurs within 15 min after CCl_4 administration to phenobarbital-pretreated animals. It has been reported that *in vivo* administration of CCl_4 [7, 8] and other chlorinated hydrocarbons [8, 9] results in inhibition of the microsomal calcium pump. The present report shows that loss of calcium from the microsomal fraction correlates with inhibition of the microsomal calcium pump when dose–response and temporal relationships are considered, and when hepatotoxicity of the chlorinated hydrocarbon is modified by pretreatment of animals with compounds that alter drug metabolism. To demonstrate that ionized calcium in cytoplasm is increased after CCl_4, we examined the activity of an enzyme that responds to changes of cytoplasmic calcium. Phosphorylase *a* activity was increased after administration of CCl_4 to an animal. These results suggest that the liver endoplasmic reticulum (microsomal) calcium pump sequesters a calcium pool *in situ* and thus provides evidence that this calcium pump participates in regulation of cytoplasmic calcium.

METHODS

Animals and hepatotoxin administration. Male Sprague–Dawley (Taconic Farms) rats with body weights of 200–300 g were used for this study. Animals were allowed free access to food and water throughout the experiments except as noted. Chlorinated hydrocarbons were administered intraperitoneally (i.p.) in corn oil. Control animals received equivalent volumes of corn oil i.p. In indicated experiments, rats were pretreated i.p. with phenobarbital (80 mg/kg) 72, 48, and 24 hr before administration of the chlorinated hydrocarbon. One group of animals was pretreated i.p. with beta-diethylaminoethyl diphenylpropylacetate (SKF-525A, 37.5 mg/kg) 45 min before administration of CCl_4. Hepatic injury was assessed by determination of glutamic/pyruvic transaminase in serum (SGPT) with a commercial kit (Sigma Chemical Co., St. Louis, MO).

Calcium determination in subcellular fractions and whole liver. Samples of liver were rinsed, weighed, dried overnight at 95°, and finally ashed for 72 hr at 600°. The ashed samples were collected in 0.1 N HCl

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containing 0.1% $LaCl_3$. Calcium associated with the homogenate and with subcellular fractions of liver was determined as described by Schmidt and Way [10]. Results for whole liver were expressed as μg calcium/g tissue (wet weight) and for homogenate or subcellular fractions as μg calcium/mg protein. Calcium was determined with a Perkin–Elmer 603 atomic absorption spectrophotometer using an acetylene–air flame.

Calcium pump determination. Calcium pump activity was determined with the microsomal fraction as described previously [7, 9]. Briefly, microsomes were prepared as the 12,500–105,000 *g* pellet from homogenates in 0.25 M sucrose. Calcium pump activity was determined with [$^{45}Ca^{2+}$] in a medium containing 100 mM KCl, 30 mM imidazole–histidine buffer (pH 6.8), 5 mM sodium azide, 5 mM ammonium oxalate, 5 mM $MgCl_2$, 5 mM ATP, 20 μM $CaCl_2$ ([$^{45}Ca^{2+}$], 1 μCi/ml) and 20–40 μg/ml microsomal protein. Protein concentrations of microsomal suspensions were determined by the Lowry method as described by Shatkin [11].

Calcium binding determination. Passive binding of calcium to the microsomal fraction was determined in the following medium: 100 mM KCl, 30 mM imidazole–histidine buffer (pH 6.8), 5 mM $MgCl_2$, 5 mM NaN_3, 20 μM $CaCl_2$ ([$^{45}Ca^{2+}$], 0.2 μCi/ml) and 200–400 μg microsomal protein/ml. At timed intervals, 0.5-ml samples were removed and processed as described for the calcium pump determination [7, 9].

Phosphorylase a determination. To determine phosphorylase *a* activity with as little influence from environmental stimuli as possible, the following procedure was rigorously adhered to. Rats received i.p. injections of CCl_4 at 8:00 a.m. Food was withheld from the animals after this time. Sampling occurred 15 min to 24 hr after CCl_4 administration. Fifteen minutes prior to the time liver tissue was sampled, pentobarbital was given in an anesthetic dose (50 mg/kg, i.p.). At the time for sampling, a midline incision was made on the abdomen of each rat and a portion of the left median lobe of the liver was rapidly frozen between aluminium blocks cooled to the temperature of liquid nitrogen. Phosphorylase *a* activity and phosphorylase *a* + *b* activities were determined by the method of Golden *et al.* [12] modified to include 10% 1,2-dimethoxyethane for determination of phosphorylase *a* + *b* as described by Uhing *et al.* [13].

Glycogen and adenosine 3′:5′-cyclic monophosphate determinations. Glycogen was determined as described by Huijing [14]. Adenosine 3′:5′-cyclic monophosphate (cAMP) was measured by the protein binding method of Gilman and Murad [15] as modified by Brostrom and Kon [16].

Statistical analysis. Statistical significance of the treatment effect upon phosphorylase *a* activity was determined by a test of the F-values following a two-way analysis of variance (ANOVA) with repeated measures of the randomized block design [17].

RESULTS

Dose–response relationship between inhibition of the microsomal calcium pump and loss of microsomal calcium after CCl_4 treatment. As previously described [9], treatment of rats with CCl_4 results in a dose-dependent loss of calcium pump activity in isolated liver microsomal fractions. Rats were treated with CCl_4, killed 2 hr later, and microsomal fractions isolated. At the maximal dose examined (3 ml/kg), CCl_4 decreased microsomal calcium pump activity 90% (Fig. 1). At lower doses (1 to 0.03 ml/kg), progressively less inhibition of the calcium pump was observed. In addition to sequestration of calcium by the energy-dependent calcium pump, calcium is passively bound to microsomal membrane components [18]. Passive binding of calcium to microsomal membranes was not altered by CCl_4 treatment (Fig. 1).

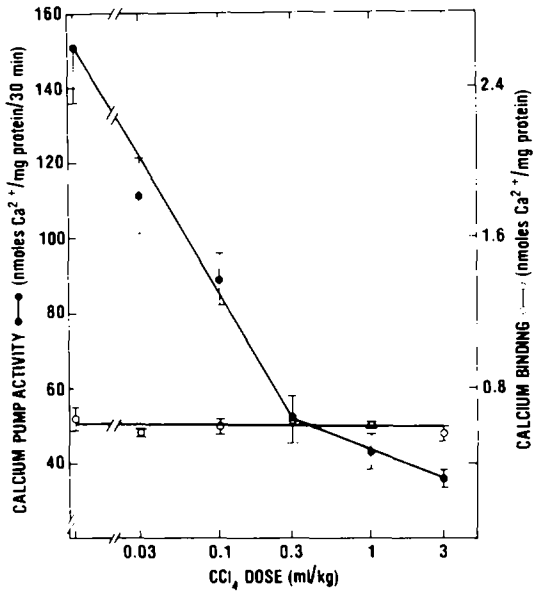

Fig. 1. Effect of CCl_4 administration to rats on calcium binding and calcium pumping by a liver microsomal subcellular fraction. Rats were treated with various doses of CCl_4 and killed 2 hr later. Liver microsomes were isolated, and passive calcium binding and active, energy-dependent calcium pumping activities were determined as described in Methods. The results shown are the mean ± S.E.M. for the determinations with five to seven microsomal preparations.

Previous reports have shown that calcium associated with the microsomal fraction is reduced significantly when the microsomal fraction is isolated from an animal treated with CCl_4 [6, 9, 19]. Loss of calcium associated with a subcellular fraction was relatively specific for a portion of the microsomal fraction (Table 1). Loss of calcium from the membrane fractions isolated from livers of animals treated with CCl_4 occurred principally in a "light" microsomal fraction sedimenting between 32,500 and 105,000 *g*. Forty-five percent of the calcium normally associated with this fraction was released after CCl_4 treatment. A "heavy" microsomal fraction sedimenting between 12,500 and 32,500 *g* released 20% of the membrane-associated calcium found in that fraction (Table 1). In contrast, previous reports have shown that calcium is accumulated by mitochondrial fractions isolated from liver of rats treated with CCl_4 [7, 20]. This was interpreted as mitochondrial accumulation of extracellular calcium leaking

Table 1. Calcium associated with liver subcellular fractions

Subcellular fraction	Calcium (μg/mg protein)	
	Control	CCl_4 treated
1,500 *g* Pellet	0.385 ± 0.054	0.393 ± 0.038
4,500 *g* Pellet	0.561 ± 0.048	1.01 ± 0.228
12,500 *g* Pellet	0.465 ± 0.051	0.446 ± 0.066
32,500 *g* Pellet	0.476 ± 0.024	0.386 ± 0.034
105,000 *g* Pellet	0.461 ± 0.079	0.253 ± 0.010
105,000 *g* Supernatant	<0.02	<0.02

Rats were treated with corn oil (control) or CCl_4 (3 ml/kg) in corn oil. One hour after treatment the animals were killed and liver samples were homogenized in 0.25 M sucrose. The indicated pellets or supernatant were isolated with the following centrifugation scheme: 1,500 *g* × 10 min, followed by 4,500 *g* for 10 min, followed by 12,500 *g* for 10 min, followed by 32,500 *g* for 10 min, and finally 105,000 *g* for 60 min. The microsomal pellet used for the calcium pump determination has been subdivided into 32,500 *g* and 105,000 *g* pellets for this determination. Calcium in the fraction was determined as described by Schmidt and Way [10]. Results are expressed as the mean ±S.E.M. for the determination on fractions isolated from four animals in each group.

through damaged hepatocellular plasma membranes. However, a portion of the calcium accumulated by mitochondria may have come from intracellular stores. At this early time point (1 hr), there was no change in total liver calcium (data not shown), and the hepatocellular plasma membrane had not been sufficiently damaged to allow leakage of cytoplasmic enzymes (as an example see SGPT in Fig. 3). A pellet (1500–4500 *g* pellet) containing mitochondria had almost twice the normal amount of calcium (Table 1).

There is a dose-dependent inhibition of the liver microsomal calcium pump and release of calcium from the microsomal fraction. When rats were treated with increasing doses of CCl_4, microsomal calcium levels and inhibition of the microsomal calcium pump were approximately linearly related between doses of 0.03 and 1 ml/kg (Fig. 2). Only at the highest dose tested (3 ml/kg) was less calcium released than would be predicted from calcium pump inhibition. In this group of animals, slightly more than half of microsomal calcium was released when the calcium pump was inhibited 90%. In this experiment, passive calcium binding assayed *in vitro* was reduced only 10% at the highest CCl_4 dose tested (data not shown).

Temporal relationship between inhibition of the microsomal calcium pump and loss of microsomal calcium after CCl_4 treatment. Shortly after CCl_4 administration, both endoplasmic reticulum calcium levels and calcium pump activity decline. During the first 4 hr after CCl_4 administration, these decreased approximately in parallel (Fig. 3). During this same time span, total calcium in the liver was not changed significantly, and significant SGPT activity did not appear in serum. Another hepatotoxin, $CHCl_3$, is reported to be a potent hepatotoxin in phenobar-

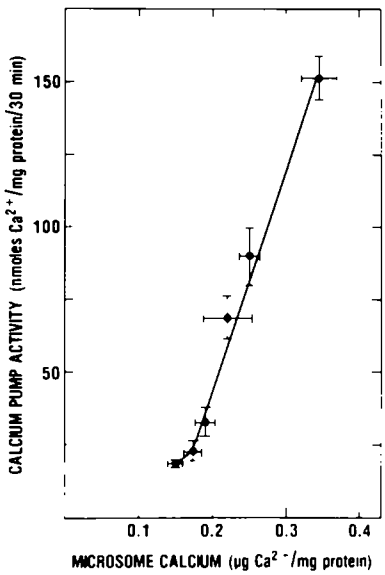

Fig. 2. Relationship between inhibition of liver microsomal calcium pump and reduction of calcium associated with liver microsomes isolated from rats treated with CCl_4. Rats were treated with CCl_4 and killed 2 hr later. Liver microsomes were isolated; calcium associated with this fraction (microsomal calcium) and calcium pump activity were determined as described in Methods. The data represent the mean ±S.E.M. for the determinations in five to seven microsomal preparations. Groups shown received the following doses of CCl_4 (left to right): 3, 1, 0.3, 0.1, 0.03 ml/kg and control, top right.

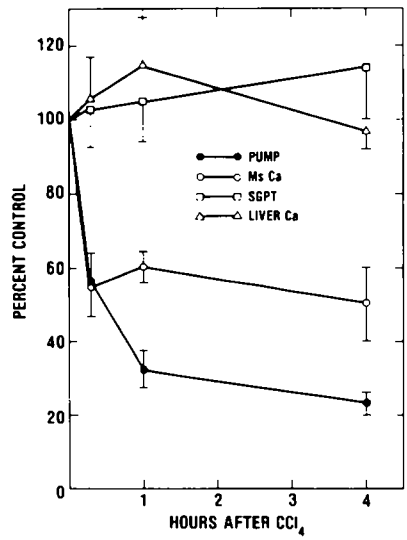

Fig. 3. Temporal relationship (hours) between inhibition of liver microsomal calcium pump and loss of microsomal calcium after administration of CCl_4 to rats. CCl_4 (1.5 ml/kg) was administered, animals were killed at timed intervals up to 4 hr, and microsomal fractions were isolated. Calcium pump activity (PUMP), microsome associated calcium (Ms Ca^{2+}), SGPT, and tissue concentrations of calcium (LIVER Ca^{2+}) were determined as described in Methods. Each point represents the mean ±S.E.M. for the determinations in preparations from five animals. Control values were: PUMP, 160 ± 15.7 nmoles calcium/mg protein/30 min; Ms Ca^{2+}, 0.358 ± 0.0212 μg calcium/mg protein; SGPT, 54 ± 3 I.U./l; and LIVER Ca^{2+}, 68 ± 7.3 μg calcium/g liver.

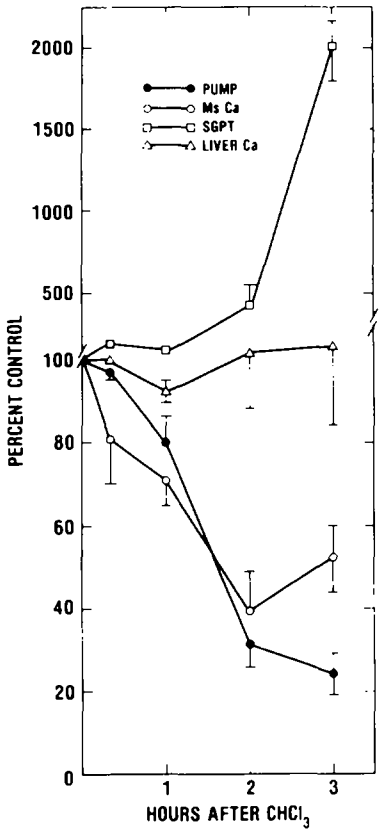

Fig. 4. Temporal relationship between inhibition of liver microsomal calcium pump and loss of microsomal calcium after administration of $CHCl_3$ to rats. Rats were pretreated with phenobarbital (80 mg/kg/days × 3). $CHCl_3$ (0.3 ml/kg) and killed up to 3 hr after $CHCl_3$ administration. Samples were isolated, and activities or concentrations were determined as described in Methods. Abbreviations on the figure are identical to those on Fig. 3. Each point represents the mean ±S.E.M. for the determination in preparations from five animals. Control values were: PUMP, 119 ± 5.5 nmoles calcium/mg protein 30 min; Ms Ca^{2+}, 0.345 ± 0.0165 μg calcium/mg protein; SGPT, 34 ± 9 I.U./l; and LIVER Ca^{2+}, 60 ± 3.5 μg calcium/g liver.

bital-pretreated rats [21]. $CHCl_3$ markedly inhibits the liver endoplasmic reticulum calcium pump and releases calcium from microsomal membranes [9]. In the phenobarbital-induced animal, the time course of calcium release from the endoplasmic reticulum paralleled loss of calcium pump activity (Fig. 4). Two hours after administration of this hepatotoxin, calcium had not accumulated in the liver and SGPT activity had not appeared in serum.

The temporal relationship between endoplasmic reticulum calcium and calcium pump activity was examined over extended time periods, for a period of 4 days after administration of a single dose of CCl_4, inhibition of the endoplasmic reticulum pump and the quantity of calcium associated with these membranes approximately paralleled each other (Fig. 5). As SGPT activity and total liver calcium decreased toward normal levels (indicating recovery from the toxic insult), calcium pump activity and calcium associated with the membranes increased toward normal values.

Effects of altered drug-metabolizing activity on chlorinated hydrocarbon inhibition of liver microsomal calcium pump activity and release of liver microsomal calcium. $CHCl_3$ and CCl_4 are activated to toxic metabolites by the cytochrome P-450 drug-metabolizing enzyme system. Induction of this enzyme system by pretreatment with phenobarbital increases hepatotoxicity of both $CHCl_3$ [9, 21] and CCl_4 [6, 22]. On the other hand, inhibition of metabolism of CCl_4 by pretreatment with compounds such as SKF-525A decreases liver damage produced by CCl_4 [23]. Alteration of the liver endoplasmic reticulum drug-metabolizing activity, and hence hepatotoxicity of CCl_4 and $CHCl_3$, has been used as another approach to correlate the effects of these agents on the microsomal calcium pump and release of microsomal calcium. Phenobarbital induction of the cytochrome P-450 system markedly potentiated both inhibition of the microsomal calcium pump and release of calcium from the microsomal fraction following $CHCl_3$ (Table 2). Similar results were observed when the effect of CCl_4 on the calcium pump and on microsomal calcium were compared in normal and phenobarbital-induced animals (Table

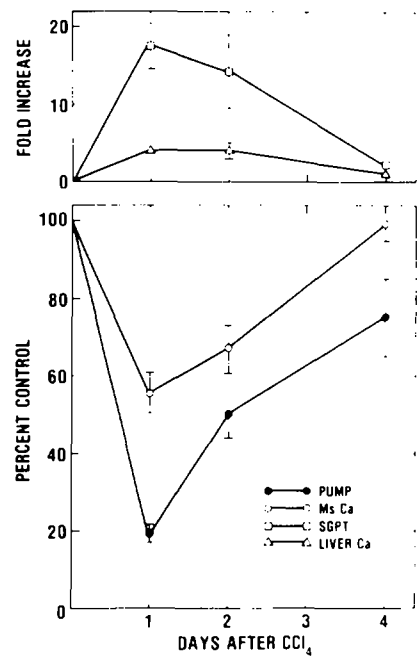

Fig. 5. Temporal relationship (days) between inhibition of liver microsomal calcium pump and loss of microsomal calcium after CCl_4 administration. Rats were treated and samples prepared as described in Fig. 3 except that animals were killed up to 4 days after CCl_4 administration. Abbreviations on the figure are identical to those on Fig. 3. Each point represents the mean ±S.E.M. for the determinations in preparations from five animals. Control values were: PUMP, 184 ± 13 nmoles calcium/mg protein 30 min; Ms Ca^{2+}, 0.375 ± 0.0105 μg calcium/mg protein; SGPT, 43 ± 10 I.U./l; and LIVER Ca^{2+}, 54 ± 3.3 μg calcium/g liver.

Table 2. Effect of phenobarbital induction on $CHCl_3$ inhibition of liver microsomal calcium pump and release of calcium from the microsomal fraction

	Calcium pump activity (nmoles Ca^{2+}/mg protein/30 min)	Microsomal calcium (μg Ca^{2+}/mg protein)
Control	124 ± 5.4	0.30 ± 0.02
$CHCl_3$	101 ± 9.1	0.29 ± 0.03
Phenobarbital control	122 ± 4.9	0.29 ± 0.03
Phenobarbital + $CHCl_3$	23.1 ± 5.3	0.15 ± 0.02

Control and phenobarbital-induced control rats received corn oil. Phenobarbital-induced animals were pretreated with daily injections of 80 mg/kg for 3 days. One day after the last phenobarbital dose, $CHCl_3$ was administered at a dose of 0.3 ml/kg in corn oil. Two hours later the animals were killed. Liver microsomes were isolated, and microsomal calcium pump activity and microsomal calcium were determined as described in Methods. Data are expressed as the mean ±S.E.M. for determinations in six microsomal preparations.

3). It was clear that, when CCl_4 hepatotoxicity was increased by phenobarbital pretreatment, the effects on both the microsomal calcium pump and on microsomal calcium were potentiated. On the other hand, when hepatotoxicity of CCl_4 was decreased by pretreatment with SKF-525A, the effects of CCl_4 on both calcium pump activity and release of calcium from intracellular membranes were attenuated (Table 3).

Effect of CCl_4 *administration on phosphorylase* a *activity*. In liver tissue from rats that received CCl_4, phosphorylase *a* levels were 33% higher as early as 30 min after CCl_4 and 50% higher by 60 min after CCl_4 (Fig. 6). By 24 hr after CCl_4, phosphorylase *a* levels remained elevated over control levels. The diurnal fluctuations characteristic of this enzyme were not altered by CCl_4 administration. The difference between phosphorylase *a* levels found in CCl_4-treated and control animals was significant for the treatment as a whole. Total liver phosphorylase activity (*a* and *b* forms) did not differ between the control and treated groups at any time point examined, except at 24 hr (data not shown). By 24 hr there was significantly less total phosphorylase activity in the liver of rats that had received CCl_4.

Changes in hepatic glycogen content and cAMP levels were investigated in liver tissue of rats that received CCl_4. To confirm that conversion of phosphorylase *b* to the *a* form actually occurred *in vivo*, liver glycogen concentrations were determined. Accelerated depletion of liver glycogen from liver of rats that received CCl_4 reflected prolonged activation of liver phosphorylase. In the control group, a steady decline of glycogen content from 40 mg/g liver to essentially zero by 24 hr was observed (Fig. 6). This is consistent with the fact that food was withheld from all animals after CCl_4 administration. Following CCl_4, liver glycogen concentration was 25% lower

Table 3. Effects of altered drug metabolism on CCl_4 inhibition of liver microsomal calcium pump and release of calcium from the microsomal fraction

	Calcium pump activity (nmoles Ca^{2+}/mg protein/30 min)	Microsomal calcium (μg Ca^{2+}/mg protein)	SGPT (I.U./l)
Control	181 ± 3.8	0.29 ± 0.02	
CCl_4	87.1 ± 6.9 (48%)	0.22 ± 0.3 (73%)	
SKF-525A	152 ± 9.9	0.27 ± 0.03	
SKF-525A + CCl_4	111 ± 9.4 (73%)	0.24 ± 0.02 (88%)	
Control	173 ± 19	0.29 ± 0.03	38 ± 9
CCl_4	136 ± 11 (79%)	0.23 ± 0.03 (78%)	31 ± 14
Phenobarbital control	153 ± 5.2	0.26 ± 0.03	33 ± 7
Phenobarbital + CCl_4	77.3 ± 6.5 (51%)	0.17 ± 0.02 (64%)	604 ± 101

Beta-diethylaminoethyl diphenylpropylacetate (SKF-525A) pretreated animals received 37.5 mg/kg 45 min before CCl_4 at a dose of 0.3 ml/kg. These animals were killed 24 hr later. Phenobarbital pretreated animals received 80 mg/kg/day for 3 days. One day later rats received CCl_4 at 0.1 ml/kg and were killed 24 hr later. Calcium in the microsomal fraction and calcium pump activity in the fraction were determined as described in Methods. Results are expressed as the mean ±S.E.M. for the determination on microsomal fractions isolated from six animals. For CCl_4 treated groups, the number in parentheses is percent of the respective control.

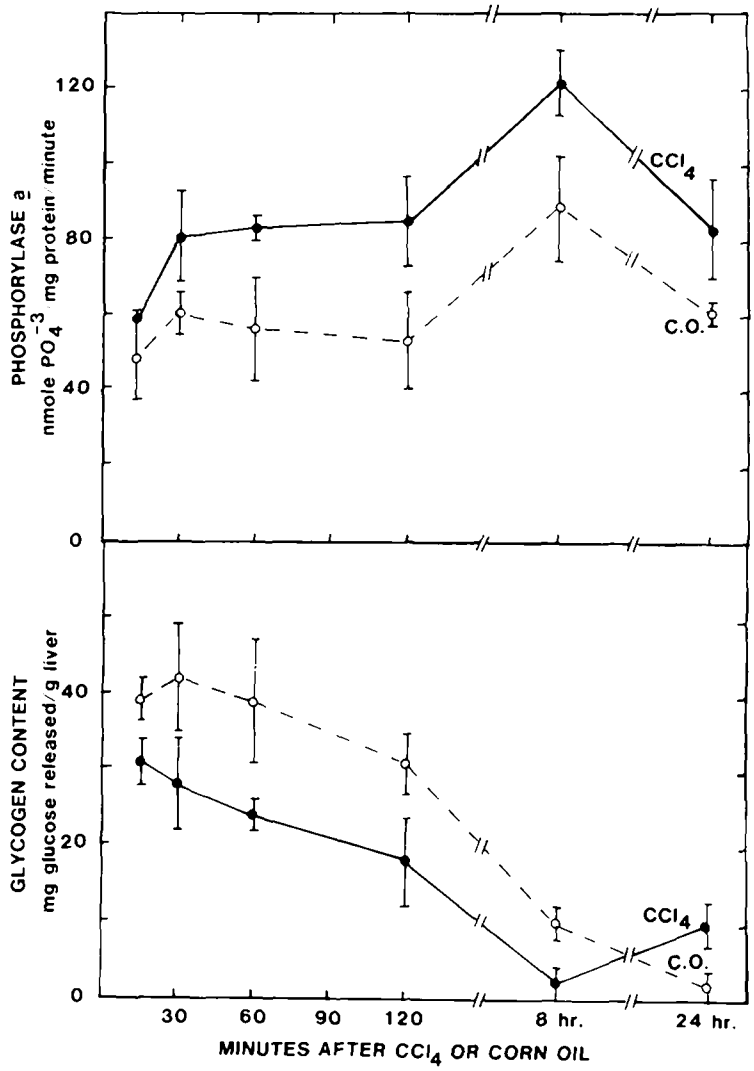

Fig. 6. (Top) Time course of appearance of glycogen phosphorylase *a* activity liver after CCl_4 (1.5 ml/kg) or corn oil (C.O.) administered at zero minutes. Data represent the mean ±S.E.M. from three to five rats at each time point. Results are expressed as nmoles glucose incorporated into glycogen/mg protein.min and are corrected for variations in total phosphorylase (*b* + *a*) activity. (Bottom) Time course of disappearance of liver glycogen after CCl_4 (1.5 ml/kg) or corn oil (C.O.) administered at zero minutes. Data represent the mean ±S.E.M. from three to five rats at each time point. Results are expressed as mg glucose liberated from glycogen/g liver. Differences between CCl_4 and C.O. are significant by a test of the F-values ($P < 0.05$) following a two-way ANOVA.

at 30 min and remained lower than in the control group until more than 8 hr after the CCl_4 dose. As with phosphorylase *a* activity, this effect was significant when considered for CCl_4 treatment as a whole. Paradoxically, glycogen content was increased slightly at 24 hr after CCl_4. Perhaps glycogen synthesis occurred in the animals recovering from CCl_4. It was observed that the stomachs of these animals contained food at this time, unlike the stomachs of the control animals. Measurement of liver cAMP concentrations demonstrated no statistically significant differences between treated and control groups at any time (data not shown). cAMP concentrations averaged 0.6 to 0.8 pmole/mg liver at all time points.

DISCUSSION

Earlier studies demonstrated reduction of the amount of calcium associated with liver ER after CCl_4 administration to an animal [6]. This suggested that CCl_4 metabolites might disrupt calcium homeostasis in liver cells. Several years later it was noted that CCl_4 administration to an animal inhibited calcium pump activity associated with liver ER [7, 9]. If these events were related, it could suggest that the liver ER calcium pump sequesters an intracellular pool of calcium in the ER. The results presented here demonstrate for the first time a correlation between CCl_4 dose and responses of both ER calcium pump activity and ER calcium levels. The results

presented here also demonstrate that this correlation existed not only when hepatotoxicity of CCl_4 was increased but also when hepatotoxicity of CCl_4 was decreased by modifying drug metabolism (Table 3).

One question raised by measurement of calcium associated with ER cannot be answered with techniques presently available. It is not entirely clear if the decrease of calcium in the ER occurs in the intact cell or is the result of a redistribution artifact after homogenization of the tissue. In this work, an attempt was made to prevent redistribution artifacts by promptly cooling small samples of liver. It should be noted that the levels of calcium reported here are similar to those found in another study in which $LaCl_3$ was added to homogenization medium to minimize redistribution [9]. This study suggests that the decrease of ER calcium observed here represents loss of calcium from this organelle *in vivo*.

Conversion of phosphorylase *b* to phosphorylase *a* provides evidence that inhibition of the ER calcium pump by CCl_4 does release an intracellular pool of calcium *in vivo*. CCl_4 administration did not change the total quantity of the enzyme present in liver, but did convert (by phosphorylation) phosphorylase *b* to the *a* form. Determination of cAMP levels allows us to conclude that, within the first 8 hr after CCl_4 administration, the conversion of phosphorylase to the *a* form was independent of a detectable increase in cAMP. These results are consistent with the interpretation that elevation of phosphorylase *a* after CCl_4 administration *in vivo* occurred in a calcium-dependent manner. Presumably this reflects an effect of the calcium–calmodulin complex upon phosphorylase kinase, the calcium-dependent kinase in the phosphorylase cascade [24].

Current evidence suggests that ionized calcium in liver cell cytoplasm is in the range of 100–300 nM [4, 25, 26]. It is likely that the liver ER calcium pump contributes to maintenance of this level of calcium ion by sequestering calcium in the ER [4, 5, 7]. A substantial amount of calcium can be released from ER by CCl_4 action. Based upon data presented in Tables 1–3 and estimates of ionized calcium in cytoplasm [4, 25, 26], it has been calculated that maximal doses of CCl_4 used in this study could increase calcium in cytoplasm by as much as 100- to 1000-fold. It is possible that such an increase of ionized calcium in cytoplasm can activate calcium-stimulated enzymes that can damage cellular membranes sufficiently to allow influx of extracellular calcium and produce cellular necrosis [27]. At the highest dosage of CCl_4 employed in this study, there is no change in total liver calcium [7, 9] or in homogenate calcium (data not presented) within 2 hr. However, calcium associated with the pellet containing mitochondria was almost doubled. At the same time, the microsomal pellet had lost calcium. Mitochondria have been shown to accumulate substantial calcium loads during the later stages of CCl_4-induced hepatotoxicity [7, 19]. It is possible that, in this experiment, at least part of the calcium lost from the microsomal fraction was sequestered by mitochondria.

In summary, the data presented here demonstrate that CCl_4 administration to rats inhibited the hepatic ER calcium pump. This in turn released calcium from the ER as reflected by decreased calcium associated with the microsomal fraction isolated from these animals. The conversion of phosphorylase *b* to the *a* form suggests that CCl_4 administration produces a physiologically significant increase of ionized calcium in the cytoplasm of the liver cell. It is possible that a prolonged elevation of calcium in these cells can initiate processes that culminate in sufficient disruption of the permeability barrier at the plasma membrane that death of the cell occurs.

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